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EFFICIENCY OF SOME CHEAP MATERIALS IN REMOVING SOME HEAVY METALS

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Abstract:

Investigation tests showed the susceptibility of bacterial isolates (Leuconostoc mesenteroids spp.mesenteroids ATCC829, Leuconostoc mesenteroids, Leuconostoc mesenteroids ssp.dextranicum, Lactobacillus casei, Lactobacillus rhamosus GG, Lactobacillus plantarum, Leuconostoc mesenteroids) to biofilm formation using titration microplates. In removing metals, it was chosen in conducting subsequent experiments, and the live mass of the two isolates Lactobacillus casei and Leuconostoc mesenteroids showed better retention of lead, cadmium, and cobalt than the biofilm of which it is composed. The results of restriction using multiple materials (sodium alginate, white sand, glass sand, sugar cane residues, activated carbon, Orbeez balls, silica gel, sponge, sunflower seed husks, date kernels, and coconut residues) showed positive results in the removal process. The restriction of adsorption-efficient bacterial cells using Orbeez balls and silica gel was shown to be the best substrate compared to other restrictive substrates.

Keywords: Leuconostoc Mesenteroids, Orbeez Balls, Silica Gel, Biofilm, Heavy Metals.

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Introduction:

Water is a natural resource, ocean sciences, conducive to obtaining organisms in the global environment.(*Roderick et al., 2006*).

Sanitation journalism in industrial, industrial, agro-industrial waste, industrial waste, sewage (*Ofomaja and Ho, 2007*). The water environment is exposed to a number of pollutants, including agricultural fertilizers, petroleum hydrocarbons, heavy metals, pesticides, thermal pollution, radiation, and other types of pollutants and heavy metals such as electric batteries, electrical industries, electronic devices, and the leather industry. Therefore, heavy metals are the most important pollutants for the water environment, such as the release of large quantities into the environment from Industrial water, which causes a number of problems for the environment (*Cheepi, 2012*). The danger of large quantities of various pollutants lies in their entry into the food chain and accumulate in the tissues of living organisms through bioconcentration processes and their effects on human health. Therefore, water pollution with heavy metals is of great concern all over the world, including identifying and classifying these metals, controlling them and evaluating their effects. Environmental treatment and its effects, monitoring and searching for scientific mechanisms to reduce it and prevent any potential effects that may occur due to this pollution (*Mohamedi et al., 2005*).

A number of technologies are already being used to clean the environment from these types of pollutants, but most of them are expensive and not effective enough to allow recovery of very dilute heavy metals present in the effluent and unsuitable for small industries (*Demirbas., 2008*).

In the absence of effective physical, chemical, or high-cost methods for treating wastewater, in addition to that, it leaves harmful secondary residues to the environment, which adds another burden to those environments (*Hag and Shakoori, 2000*), it will be necessary to find other methods, as it is found that biotechnologies are The successful and appropriate alternatives due to their low economic cost, treatment of low concentrations of heavy elements, and the lack of sedimentation resulting therefrom (*Volesky and Naja,2005*)and it deals with various concentrations either through Bioaccumulation or Bioadsorption (*Preetha and Viruthagiri, 2005*) and through the use of effective biological systems such as the ability of plants and their waste to remove pollutants from the environment (*Cheepi, 2012*). The majority of microorganisms through the bio-adsorption process treat a wide range of contaminated water with different concentrations of heavy metals. (*Swami and Buddhi, 2006*) indicated that Gram-positive and Gram-negative bacteria possess complex structures in the components of their cell wall and that they have an important role in the removal of organic and inorganic materials. It possesses physiological properties, through the production of enzymes and secondary metabolism processes, or through the accumulation of pollutants in the wall of bacteria positive for chromium stain due to its thickness, or through the flow technique in the wall of chromium-negative bacteria, depending on the concentrations and types of minerals and the physical and chemical characteristics of water.

Polymeric natural matrices such as agar, carrageenans, and alginate were used to restrict microorganisms to them for bioremediation, as well as industrial matrices such as alcohol, polyvinyl alcohol, and polyuresin (*Partovinia et al., 2013*), and renewable materials of plant origin (*Guimaraes et al., 2009*) to eliminate the problems of waste products for the environment. By accumulating it and reducing the use of expensive materials for restraint (*Karnitz et al., 2007*), as well as sugar cane, rice husks, barley straw, cotton, wool, wood residues and animal materials *(Angelova et al., 2011*).

Materials and method of work:

Preliminary examination of the efficiency of bacterial isolates in biofilm formation:

The ability of the bacterial isolates to form biofilms was tested using the MTP microtiter plate method (*Bose et al., 2009; Salman & Khudair 2015*) using 96-hole plates with a flat bottom well.

Estimation of the residual concentration of heavy metals using the atomic absorption spectrometer

The heavy metal solutions were added to glass flasks containing 100 ml of the sterile MRS liquid medium . The media were inoculated with 1 ml of the bacterial inoculum prepared in (containing 3 x 10^8 cells / ml). The residual concentration of heavy metals in the medium for all previous experiments was estimated using the atomic spectrometer (Annex 5) by taking 10 ml from each beaker and passed through membrane filters The size of the openings is 0.45 micrometers (*APHA, 1998; Saxena, 1998*), and the removal efficiency was estimated by calculating the percentage of removal according to the following equation:

Removal percentage (%) = (element concentration before removal - element concentration after removal) / (element concentration before removal) x 100.

Preparation of base materials for the restriction process

Restriction by sodium alginate sequestration technique:

The bacterial suspension was mixed with a 4% sodium alginate solution at a ratio of 1:1 for 15 minutes, then the mixture was added in the form of drops to a sodium chloride solution (0.1) surrounded by ice with a medical syringe to obtain drops of approximately 3 mm. The balls were left for 20 minutes to harden well. It was then filled into the sterile glass column, passed the items under examination, and collected the product to measure the amount of removal.

White sand: This material was used after washing it several times with distilled water and then drying it until the weight is stable.

Activated carbon:

Activated carbon (2) was washed three times using distilled water, and 6 g of it was taken and activated in the oven at a temperature of 160 m for 24 hours (*Dianati-Tilakietal., 2004*).

Date stones:

Washing the date stones with regular water, then with distilled water to get rid of impurities, and then dry them in the oven for 24 hours at 140 C.

Sponge:

The sponge was used as a porous backing material and cut into medium-sized pieces to increase the surface area, then washed three times with sterile distilled water and added 6 g of it per 100 ml of sterile liquid media.

Glass sand:

Glass sand was obtained from the Ramadi Glass Factory, then washed with distilled water three times and dried at 160 m for one hour. 6 g of glass sand was added to 100 ml of liquid medium.

Sunflower seed husks: after washing them three times with a size of 6 gm, they were dried at a temperature of 160 m / h.

Colored Orbeez balls material: sterilize 6 gm in an autoclave at 121 C for 15 minutes at a pressure of 15 pounds / inch2.

Coconut residues: it was washed several times, and the oil was disposed of by the cold method, and 6 gm was taken from it.

Silica gel:

They are in the form of small bags that are found inside packaging boxes and contain balls, and these balls are a granular material that has a glassy luster, is porous, and it is a form of silicon dioxide that is industrially created from sodium silicate. It was used because it is a cheap material, as 6 grams of it were weighed and then washed three times with water. distilled, sterilized by autoclave and used as a binder.

Sugar cane residues:

Sugar cane waste was obtained from the sugar processing plant in Amarah, broken into small pieces, washed with distilled water three times, then weighed 6 gm, and autoclaved.

Restriction of bacterial cells and biofilms to substrates:

Each of the restriction materials was weighed and added to glass flasks containing 100 ml of the previously prepared liquid culture medium separately, the suspension was added to it and incubated at 37 °C for a period of 24 hours once and at 37 °C for a period of 14 days again, and mineral elements were added to it so that the final concentration of minerals became In the medium was 10 mg/L and incubated for half an hour and filtered with fine membrane filters with aperture size of 0.45 micrometers, then the percentage of the remaining heavy metal elements was estimated by the atomic absorption apparatus.

Results and discussion

Choosing the most efficient bacterial isolates for removing heavy metals:

The ability of the three bacterial isolates *Lactobacillus casei*, *Leuconostoc mesenteroids* and *Lactobacillus rhamosus* GG (*L.casei, L.M,* and *L.GG*) was tested separately to remove lead, cadmium, and cobalt separately under the same conditions, in order to select the two most efficient isolates for removal and adopt them in subsequent experiments.

Use a concentration of 10 mg/L for each of lead, cadmium and cobalt in an incubation period of 14 D, 24 h at a temperature of 37 C.

Figure (1) shows that the removal efficiency of L.M. (*Leuconostoc mesenteroids*) bacteria for the three metal elements (cadmium, lead, cobalt) is (77.165, 91.317, 90.848)%, respectively, when incubating for 24 hours, but for the period of incubation for 14 days, it was (14.584,37.131, 72.313)%, respectively, while *Lactobacillus rhamnosus GG* (LGG) recorded a removal efficiency of (37.598, 78.31, 71.909)%, respectively, for the bacteria that were incubated for 24 hours, while it was (13.906,17.303,40.691)%, respectively, in 14 days. The results showed that the removal efficiency of *Lactobacillus casei* (L.C) was (55.946, 85.42, 83.452)%, respectively, for the bacteria that were incubated for 24 hours, while it was (23.359, 48.515, 37.033)%, respectively, in 14 days.

Variation in the removal efficiency of heavy metals by microorganisms is due to the difference in the chemical composition of the cell wall and its characteristics of active groups as well as the biofilm and its composition and the type of polysaccharides in it, which leads to a difference in the adsorption capacity and the difference in the ability of microorganisms in the efficiency of heavy metal removal (*Ilhanet al, 2004*)The more binding sites are available, the more efficient the removal process will be (*Vijayaraghavan and Yun, 2008*) EPS polysaccharides play a major role in binding metal ions to hydroxyl, carboxylate and phosphate groups which increases the binding sites for heavy metals (*Landersjo et al, 2002*). These groups It is one of the most active groups in the cell wall of lactic acid bacteria (*Wierzba, 2015*).

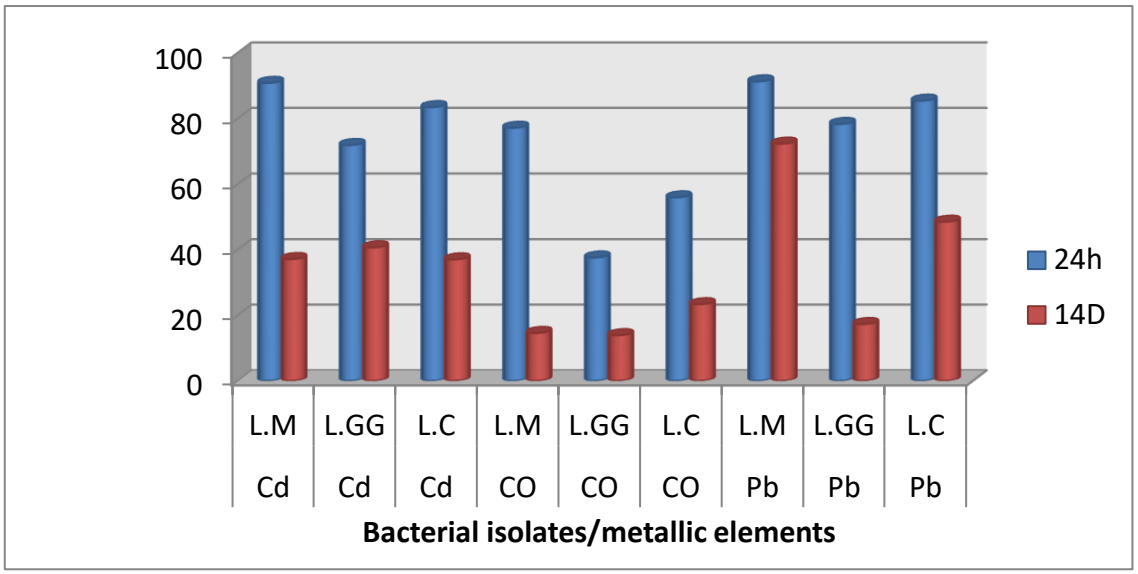

Figure (1) Removal efficiency of heavy metals (cadmium, cobalt and lead)using the biomass and biofilm of the isolates under study.

Selection of materials restricted for removal:

The economic aspect has an important impact in any process, especially in heavy metal removal operations at the applied level. It should be noted that the use of high-cost materials and pure materials works to increase the cost when applying, so the tendency was to use cheap and available materials in order to achieve economic feasibility (*Annex 7*).

Figure (2 and 3) shows a comparison between the materials used to restrict bacterial cells and the biofilm of *Lb.casei* bacteria to remove cadmium and lead. (83.068)% and (84.556)% for the elements cadmium and lead, respectively, while the percentage for removing sunflower seed husks, date kernels and silica gel was (0.50.018, 63.621 50.758)%, respectively, for cadmium and (54.267,74.889,77.884)%, respectively, for element Lead .

The materials used in the technique of restriction by determining the movement of microbial cells or their biofilm by adsorption on the surfaces of supporting materials may be using inorganic materials such as sand, clay, and coal, and may be organic materials of a polymeric nature that are used directly after their preparation, such asalginate, acarose, collagen, and others (*Veglio and Beotchini 1997;Mehta and Gaur, 2005*).

The restriction method was also used by stabilizing biomass because of its advantages compared to suspended biomass, such as increased shelf life, ease of preservation, and resistance to environmental conditions. Granulated activated carbon, crushed activated carbon, wood chips, and sponge cubes were used (*Girijan and Kumar, 2019*).

Bacterial cells of *Bacillus subtilis* were bound to several materials such as sponges and clay separately to remove nickel (*El-Sersy et al., 2007*).

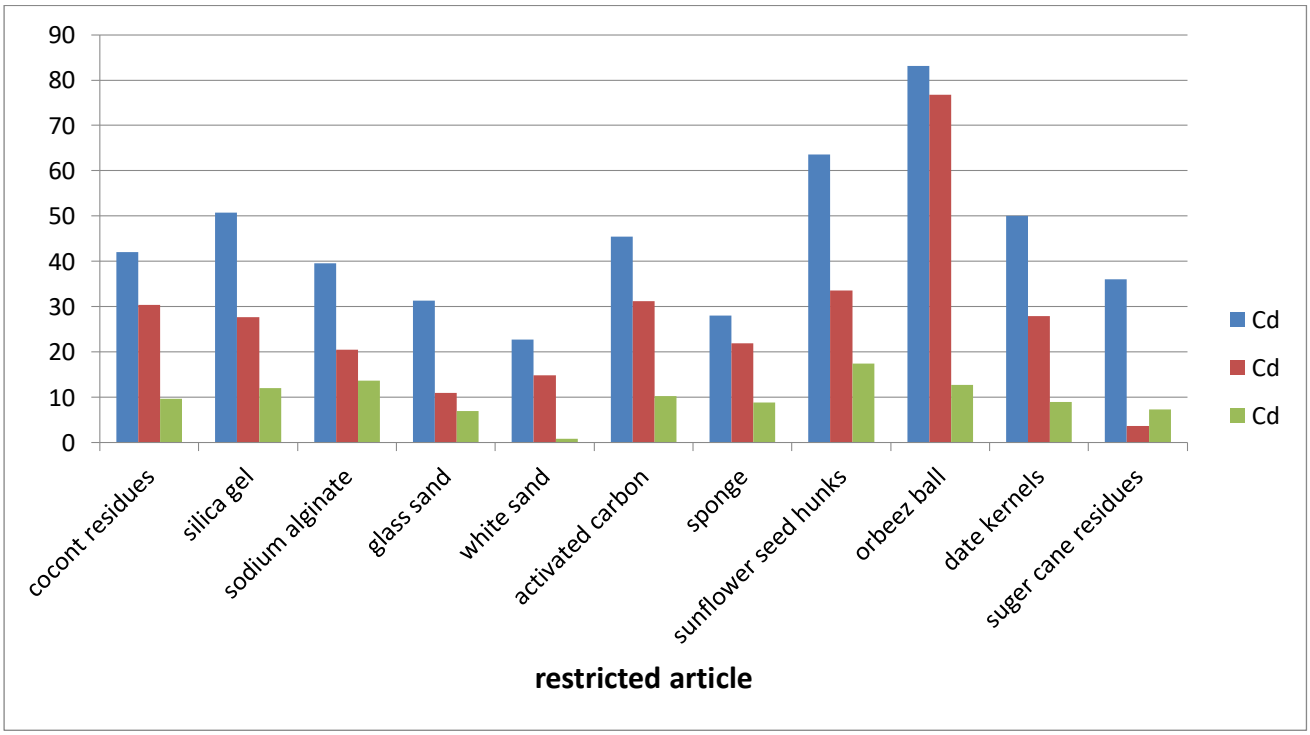

Figure (2) Substances bound to live cells and the biofilm of *Lb.casei* bacteria in the removal of cadmium

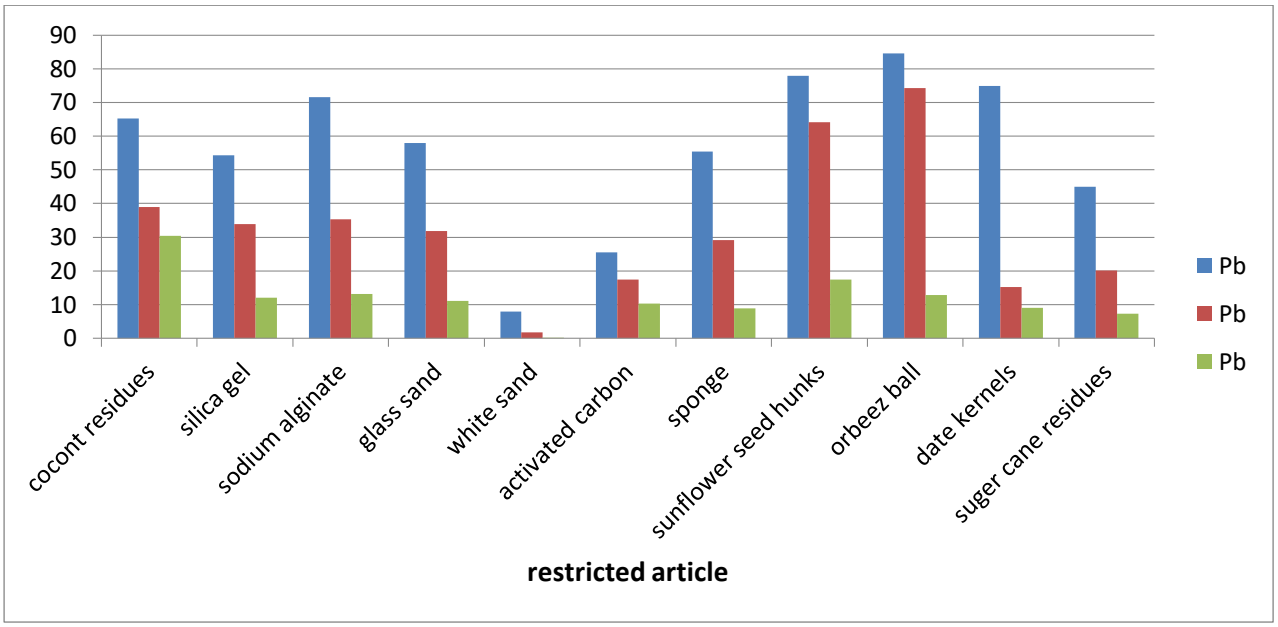

Figure (3) Substances that bind live cells and the biofilm of *Lb.casei* bacteria in removing lead

The results shown in Figure (4) and (5) indicated that the restrictive bacterial cells of the bacterial isolate *Leu.mesenteroides* were superior to the restrictive biofilm composed of this isolate in the biological removal of cadmium and lead. Silica gel, Orbeez balls, and coconut oil were the most important materials. The restricted effect was followed by date seeds and sunflower peels, respectively.

The bacterial cell wall of lactic acid bacteria contains a large number of functional groups with a negative charge, especially carboxyl and phosphoric (*Halttuen., 2007*). Also, the extracellular polysaccharides (EPS) play an important role in binding heavy metals, which are sensitive even at low metal concentration (*Gupta, and Diwan., 2017*), and it can be used again

after the process of adsorption and washing of adsorbed metal ions, which is environmentally safe and low cost (*Cai et al., 2013*).

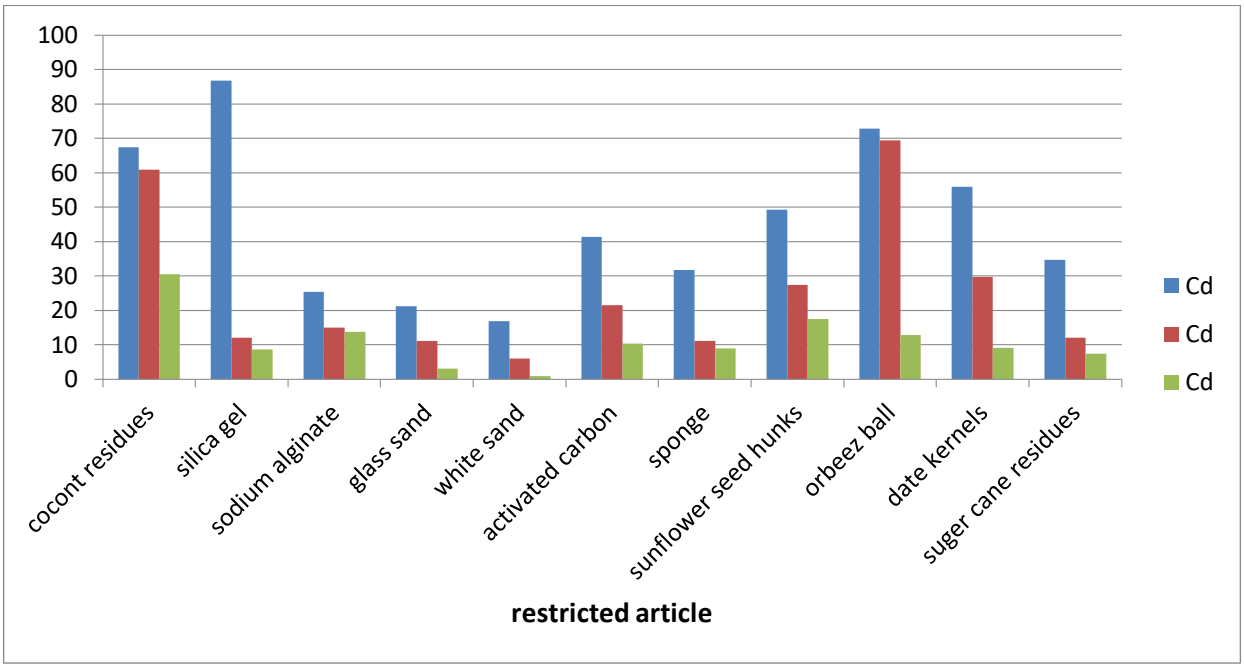

Figure (4) Substances bound to live cells and the biofilm of *Leu.mesenteroid* bacteria in the removal of cadmium

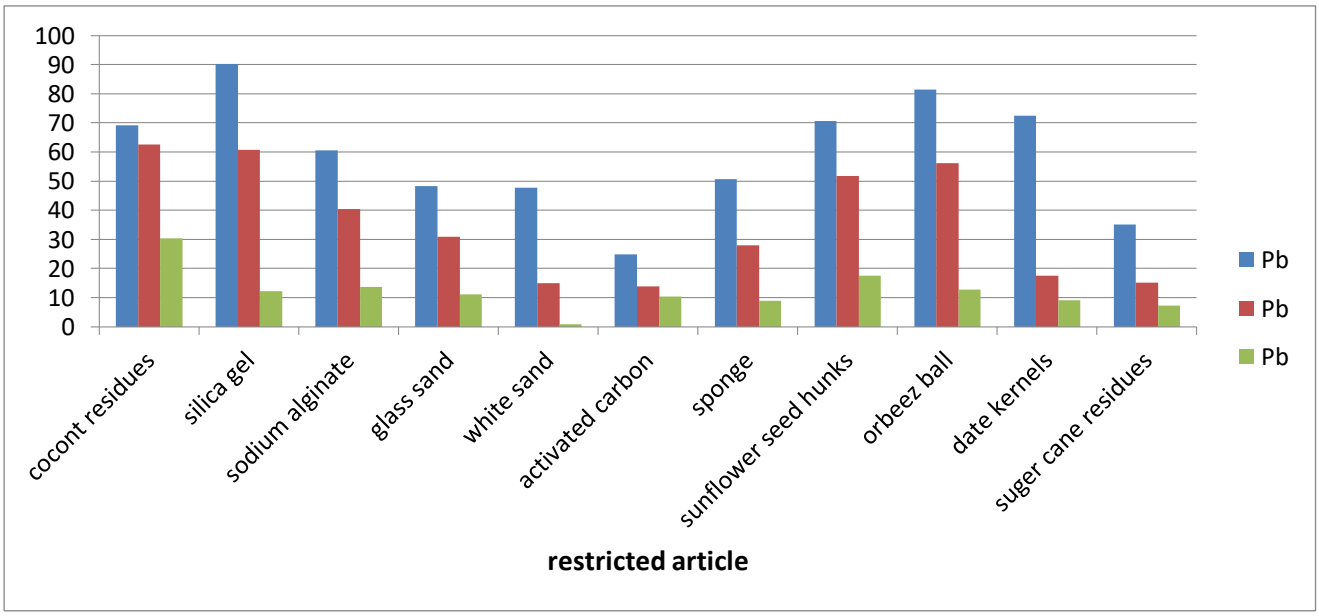

Fig (5) Substances that bind living cells and biofilms of *Leu.mesenteroid* bacteria in removing lead

Conclusion:

In this study, bacterial isolates of lactic acid bacteria with the ability to remove cadmium and lead ions were obtained, and the bacterial isolates *Leuconostoc mesenteroides* and *Lactobacillus casei* were efficient in adsorption of cadmium and lead ions than other isolates for the removal process. And after - restriction of bacterial cells that are efficient in adsorption using Orbeez balls and gel Silica as the best base material compared to other bound base materials.

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